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GENETICS AND ASEXUAL REPRODUCTION OF THE SEA ANEMONE *METRIDIUM SENILE*

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One problem in gaining an understanding of the mechanisms of natural selection centers around the difficulty in observing the dynamics of genetic processes. There are only a few studies where natural selection has been observed in the process of altering the genetic composition of natural populations. Probably the most important reason for this lack of information is one of time. Most population samples taken for genetic studies are collected only once; rather few studies have attempted to follow changes in gene frequencies with time. The classic studies of chromosomal polymorphism in *Drosophila* by Dobzhansky and co-workers (see Dobzhansky, 1970) are an outstanding exception.

There have been attempts to add a temporal dimension to genetic studies by indirect means. For example, it often is possible to gain approximate indication of the relative ages of organisms by their sizes. Boyer (1974) for *Mytilus edulis* and Koehn, Turano, and Mitton (1973) for *Modiolus demissus* have shown differences in gene frequency with size, an indicator of age in bivalves. Tracey, Bellet, and Gravem (1975) have shown similar differences in *Mytilus californianus*. While differential survival of genotypes is one potentially important component of fitness, it may not imply differential reproduction of genotypes. In addition, studies like these, especially involving animals with widely dispersed pelagic larvae, cannot rule out the possibility that differences in gene frequency with size class are the result of derivation of the size classes from different founder populations with different gene frequencies. In fact, Tracey *et al.* (1975) interpret their data on *M. californianus* as a product of this kind of population structure. Parallel variation between ecologically similar species, such as that documented by Koehn and Mitton (1972), adding differences between size classes, would be more convincing.

Metridium senile (L.) is a conspicuous sea anemone occupying rocks and pilings in protected and semiprotected habitats along the New England coast. The species exhibits color polymorphism, with individuals ranging from white to brown and red; the colors are produced by combinations of melanin and carotenoids (Fox and Pantin, 1941; Fox, Crozier and Smith, 1967). Sexual reproduction is a feature of the life cycle with eggs and sperm shed freely into the surrounding medium (Gemmill, 1920). In the Woods Hole region spawning occurs during the summer months, and the species is dioecious (Costello, Davidson, Eggers, Fox, and Henley, 1957, cited in Campbell, 1974). Asexual reproduction by pedal laceration is also prominent, commonly leading to large aggregations of identically colored individuals (also Torry, 1902).

Asexual reproduction, coupled with the sessile existence of the adult, are special features of the life cycle of *M. senile* which make it feasible to add a temporal dimension to genetic studies of the species, even when sampling is possible at

only a single time. Since long distance dispersal of new, asexually produced polyps is unlikely, and since each clone is produced from a single settling planula larva, it is possible to measure potential differential proliferation of genotypes under local natural conditions. Genotype distributions of successful planulae can be determined, since they will be reflected by the genotypes of clones; and the success of each established anemone can be measured by the numbers of monoclonal individuals that it has produced since settling. Of course, this ignores large portions of the life cycle which are probably also subject to intense selection, most prominently as a result of larval and immediate post-settling mortality. Further, Williams (1975) has recently emphasized the importance of studies on organisms with both sexual and asexual phases. This study takes advantage of the special features of the life cycle of *M. senile* to investigate dynamic aspects of genetic structure during asexual reproduction.

Materials and Methods

Methods of collection

Animals were collected from rocks, pilings, and the shells of *Mytilus edulis* by carefully scraping the foot loose with a thin spatula or penknife. They were returned to the laboratory in jars of sea water and were held for electrophoresis on water tables supplied with continuously running sea water.

Electrophoresis

Horizontal starch gels were cast from 13% (W/V) Sigma starch using covers and slot formers. The buffer system was the discontinuous lithium hydroxide system of Selander, Hunt, and Yang (1969). Gels were cast two hours before use, since prolonged aging (*e.g.*, overnight) produced unacceptable gels. Small samples of pedal disk or column of individual polyps were ground in approximately 0.5 ml of 0.1 M tris, pH 7 and centrifuged for 20 minutes at top speed in a clinical centrifuge. About 30 μl of the supernatant were loaded into each sample well, and 350 volts were applied for 5 hours. After electrophoresis, gels were sliced and stained for phosphohexose isomerase (PHI) in the following mixture: 10 mg fructose-6-phosphate, 100 mg $MgCl_2$, 10 mg NADP, 4 mg phenazine methosulfate (PMS), 20 mg MTT tetrazolium, and 20 units glucose-6-phosphate dehydrogenase to 100 ml in 0.1 M tris, pH 8. Substrates and enzymes were purchased from Sigma. Buffer components were standard reagent grade.

Locations

Three locations on Cape Cod, Massachusetts, were chosen for study.

Woods Hole Oceanographic Institution Dock. This location is on the south side of Cape Cod, an area strongly influenced by the warming of the Gulf Stream during the summer. Anemones were collected subtidally on the shells of mussels, which were gathered from an area approximately 1 m^2. No analysis of clonal

association (see Barnstable) was attempted, although individuals on a single mussel tended to be of like color, suggesting some asexual reproduction.

Cape Cod Canal. Animals were collected intertidally from rock surfaces and mussel shells found on the north jetty at the eastern end of the canal. This is a relatively high energy environment, due to currents and wave action in the canal. Consequently, animals were found in relatively protected locations, usually in the crevices among the large rocks constituting the jetty. On the first occasion animals were collected without reference to specific station or location with respect to other animals. Although detailed mapping of individual polyps for clonal analysis was technically not feasible because of close working quarters between the rocks, on a subsequent collecting trip animals were kept separate according to the rock from which they were collected to gain some indication of patchiness of distribution of genotypes. There was no appreciable color polymorphism observed at this location; all animals collected were brown.

Barnstable Town Boat Harbor. Barnstable Harbor is a protected bay on the north side of Cape Cod. The town boat harbor is a deep sloping basin lined with large rocks. Anemones were found near the low tide mark attached to these rocks and to dock pilings. Animals were generally large (> 5 cm pedal diameter), and there were sizable associations of identically colored individuals, indicating asexual reproduction. There was also a high frequency of diglyphic animals, a feature produced by asexual reproduction by pedal laceration when the piece pinched off includes a directive mesentery (Hahn, 1905).

Clonal assessments at Barnstable

Initial collections were made from two restricted stations (one or two rocks at each station), and animals collected at the same station were pooled for genetic analysis. During subsequent collections the relative positions of individual polyps were sketched by an assistant as the animals were removed from the substratum. In order to ensure complete sampling of clones, all visible animals at each station were collected. Individuals were kept separate by map location until electrophoresis could be performed.

In this population of *M. senile* it was possible to estimate clonal limits by analyzing the patterns of distribution of colors and PHI genotypes. While the mode of inheritance of color pattern is not understood, it is clear that color in *M. senile* is constant over considerable periods of time, if not the entire life of the polyp; it apparently cannot be modified by diet in spite of direct derivation of carotenoids from food (Fox *et al.*, 1967). The supposition that color is indeed genetic, rather than an environmentally induced character, is further reinforced by the occurrence of large aggregations of identically colored anemones that are often accompanied on the same rock or piling by a group of an entirely different color. A similar situation is observed in *Anthopleura elegantissima* on the Pacific coast (Francis, 1973a). For present purposes color is assumed to be constant for a given clone, and probably genetically determined. By using a combination of color, location, and PHI genotype, clone sizes and distributions were determined from the maps made in the field.

Animals were considered to be members of the same clone if, and only if, they met the following four criteria: if they occurred on the same rock or piling,

if they were the same color, if they had the same PHI phenotype, and if they were not entirely separated from a similar group by a large aggregation of a different constitution (*i.e.,* assuming that movement through another clone of closely spaced individuals is unlikely and that separation did not occur prior to the proliferation of the intervening clone). Of course, this also assumes that there is no nonelectrophoretic variation for PHI. In one case, a large individual was assigned to a clone on another closely adjacent rock because it shared an unusual color phenotype (tan with brown freckles on the column) with the larger group on the next rock.

While it is possible that there are some errors in assignment to clones by this method, they should be few, since the probability of joint occurrence of the four conditions is low unless the animals so assigned are indeed monoclonal. (An estimate of the maximum probability of error in assignment can be made as follows. There were a total of nine rocks and pilings examined in the study, so the probability of being on any of them at random is 1/9 or 0.111. The most frequent color at Barnstable is brown, and the random probability of being brown is 0.784 (120/153). The most common PHI genotype is f/s, which occurred with a frequency of 0.516. On these three conditions alone, the joint probability is the product of the independent probabilities, so $P = 0.045$. This being the maximum probability of joint occurrence, it seems certain that $P < 0.05$, overall.) This kind of analysis made possible inferences about relative propensities of genotypes to proliferate, since the original distribution of genotypes of successful planulae is known (each clone being produced by a single planula larva), as is the composition of the clones at the time of sampling.

Results

Three PHI phenotypes were observed from all collecting locations. These patterns consisted of two single banded classes, one designated "fast" on the basis of electrophoretic mobility; the other was designated "slow." The third pheno-

Table I

Gene frequencies and zygotic distributions from three localities studied for phosphohexose isomerase variation in Metridium senile, *including χ^2 analysis for goodness of fit to Hardy-Weinberg expectations. Expected numbers shown in parentheses; s.e. = $\sqrt{(pq/2N)}$.*

Location	N	Gene frequency f(±s.e.)	Genotypes			$\chi^2_{[1]}$	P
			f/f	f/s	s/s		
Woods Hole	90	0.789 ± 0.030	53 (56)	36 (30)	1 (4)	3.61	<0.10
Cape Cod Canal (all individuals)	200	0.845 ± 0.018	155 (142.8)	28 (52.4)	17 (4.8)	43.29	<0.001
Cape Cod Canal* (without B-2)	157	0.917 ± 0.016	132 (132.1)	24 (23.8)	1 (1.1)	0.01	<0.95
Barnstable† (clones)	27	0.685 ± 0.063	12 (12.7)	13 (11.7)	2 (2.7)	0.36	<0.70
Barnstable‡ (mapped individuals)	153	0.716 ± 0.026	70 (78.4)	79 (62.3)	4 (12.4)	11.07	<0.001
Barnstable (all individuals)	245	0.678 ± 0.021	93 (112.5)	146 (107)	6 (25.5)	32.50	<0.001

* Canal data analyzed without individuals from station B-2, which had a large number of s/s animals.
† Analysis of clones as the genetic individual. Clones were determined as described in the text.
‡ Barnstable analysis considering only those individuals that were mapped during collection. These individuals make up the clones analyzed in the previous line of the table.

type consisted of three bands, one corresponding to the fast band, another to the slow band, and a third intermediate band equidistant between the other two. This pattern suggests two alleles segregating at a single locus with the enzyme having a dimeric structure and random association of subunits, as is commonly observed for PHI (Wilkins and Mathers, 1974 and references therein). Following standard practice, it can be assumed that the phenotypic classes reflect the genotypes of the animals, which are designated f/f and s/s for the homozygotes and f/s for the heterozygotes. No rare alleles were observed. The gene frequencies and genotype distributions, together with analysis for goodness of fit to Hardy-Weinberg expectations, appear in Table I.

Woods Hole

The "fast" allele is the most frequent in Woods Hole, as it is in the other locations (Table I). There is a slight excess of heterozygotes over Hardy-Weinberg expectations, but the excess is not statistically significant.

Cape Cod Canal

If the data from all individuals collected at the canal are pooled and analyzed for departure from Hardy-Weinberg expectations, there is a striking deficiency of heterozygotes (Table I). Such a deficiency is commonly produced when separate populations are pooled and analyzed as one (the Wahlund effect). That an analogous phenomenon may be operative here is supported by the observation that this departure is produced as the result of a large aggregation of s/s individuals found on a single rock (station B-2). To emphasize this point, when the data from station B-2 are omitted from the analysis, the population is virtually in Hardy-Weinberg equilibrium (Table I). While detailed mapping was not possible here, station B-2 at the canal contained the only substantial aggregration of s/s individuals observed during the entire study of 535 polyps, suggesting monoclonal origin. Not surprisingly, the omission of the station containing most of the s/s individuals (16 out of 17 observed at the canal) causes a marked shift in the apparent gene frequency (Table I).

A further indication of the patchy distribution of genotypes here is offered by analysis for heterogeneity of distribution of genotypes among the stations where animals were collected in groups according to the rock from which they were removed. A rows by columns G-test (Sokal and Rohlf, 1969) reveals significant heterogeneity in genotype distribution among stations, either with station B-2 ($G = 48.84$, 8 d.f., $P < 0.001$) or without station B-2 ($G = 18.74$, 6 d.f., $P < 0.005$). There is also marked heterogeneity of distribution of gene frequencies ($G = 34.32$, 4 d.f., $P < 0.001$). By analogy with Barnstable (see below), this probably indicates that cloning is a prominent feature at the canal. Mapping of the sort carried out at Barnstable will be required to settle the question, but such analysis will require another polymorphic locus if the same degree of confidence in clonal assignments is to be achieved, since there is no observed color polymorphism at this location. The somewhat confusing picture that emerges from the

Cape Cod Canal emphasizes the value of the kind of analysis that was possible at Barnstable.

Barnstable Town Boat Harbor

The microdistribution of genotypes and clonal assignments for the five mapped collecting stations in Barnstable Harbor are shown in Figures 1–5.

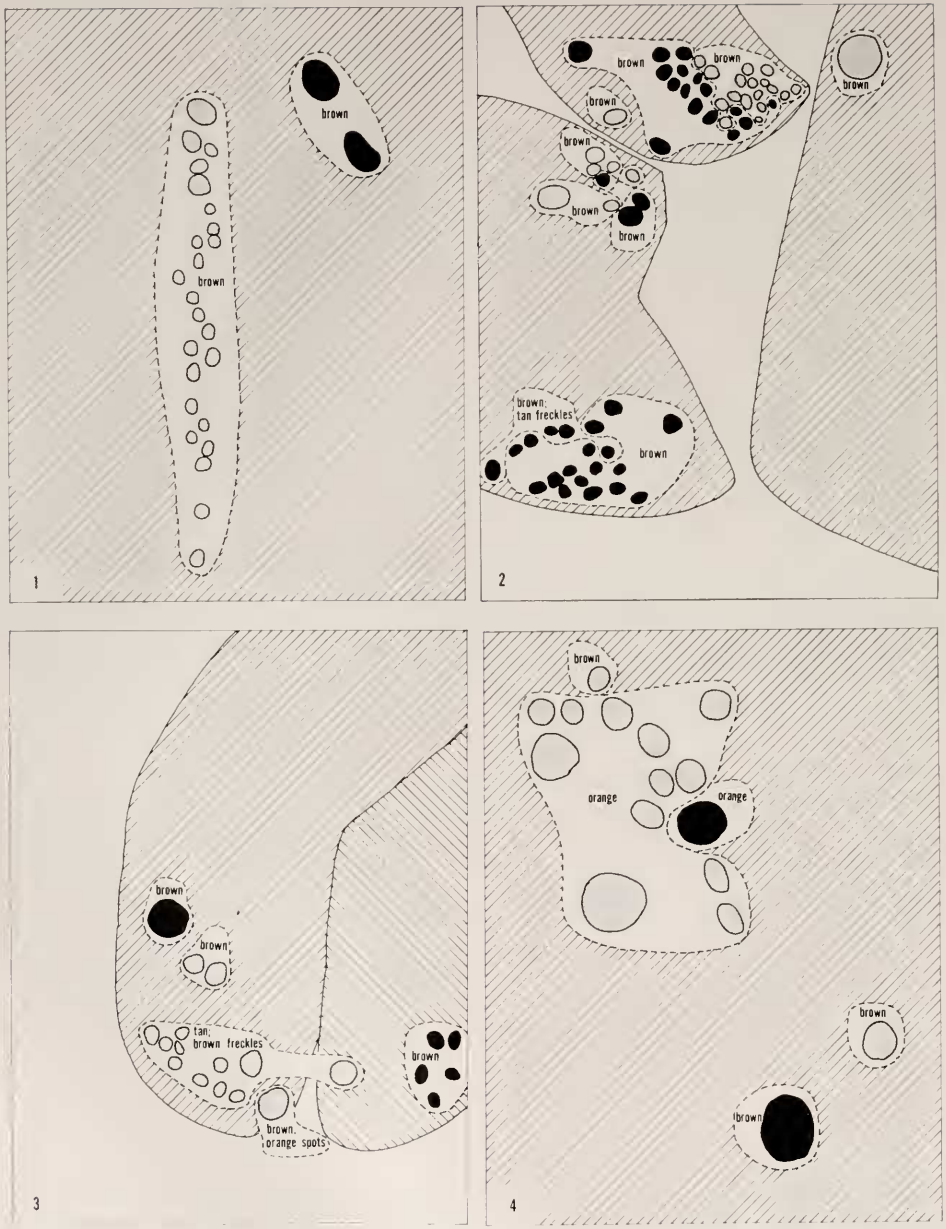
Genotype frequencies of successful larvae do not depart significantly from Hardy-Weinberg expectations (Table I). This can be inferred because the clonal boundaries can be determined and each clone results from a single larva settling from the water column. Furthermore, there is no significant heterogeneity among the five mapped stations in the distribution of genotypes at the time of establishment of successful polyps ($G = 3.14$, 8 d.f., $P > 0.90$). That is, the larvae appear to be successful in establishing new polyps at random with respect to PHI genotype.

When the analysis for goodness of fit to Hardy-Weinberg expectations is carried out using the individual mapped polyps as the genetic unit, there is an extreme departure from expectations (Table I), resulting from an excess of heterozygotes at the expense of both homozygotes. The homozygote deficiency is more pronounced for the s/s individuals. However, the fact that newly established anemones do not depart significantly from expectations and that the clones they produce do depart significantly may not imply that the two genotype distributions differ significantly from each other. In fact, the two genotype distributions (clones *vs.* polyps making up the clones) are homogeneous ($G = 1.31$, 2 d.f., $P < 0.70$). The present suggestion of heterozygote excess is considerably weakened by the homogeneity of the two distributions as they presently stand. Further, analysis of variance for an association of clone size with PHI genotype reveals no significant association ($F_{[2, 24]} = 0.316$; $0.50 < P < 0.75$).

However, there is significant heterogeneity among the stations in the distributions of genotypes when individual polyps are considered ($G = 54.47$, 8 d.f., $P < 0.001$), which is precisely what would be expected with random establishment of clones (as indicated by homogeneity of clonal genotype distribution with station) and subsequent differential proliferation of genotypes without significant movement of the progeny. While the collections from the first two Barnstable stations did not involve mapping or exhaustive collection of polyps, pooling of all animals collected in Barnstable, both those mapped and those simply collected by station, only confirms the general picture of heterozygote excess (Table I) and heterogeneity of distribution of genotypes ($G = 70.09$, 12 d.f., $P < 0.001$).

Discussion

The tacit assumption so far has been that the individual polyp rather than the clone is the unit upon which selection acts. It is clear that there is cooperation among clonemates in some aggregating anemones, and that the clone is, in fact, the ecological individual. For example, *Anthopleura elegantissima* creates boundaries between clones that are kept free of nonclonemates by aggressive behavior, presumably to reduce competition between clones. Further, if anemones are mixed in the laboratory, they will segregate into clone-specific groups, and there is evidence of cooperative feeding in the wild (Francis, 1973a, b). There is no



FIGURES 1–4. Distributions of clones and genotypes at collecting stations in Barnstable Town Boat Harbor. Shading indicates PHI genotype, filled animals being f/f, stippled f/s, and open s/s. Clonal boundaries are indicated by dashed lines, and the color of each clone is indicated within the clonal boundary.

present evidence for this kind of behavior in *Metridium*. Clones frequently sit side-by-side in the field without any evidence of separation, and it is not uncommon to see members of one clone that have moved short distances into the middle of an adjacent clone (Figure 2). Further, there is no apparent preference for clonemates to stay especially close to one another (Figures 1, 4, and 5), and there is little opportunity for cooperation in feeding in an animal that is apparently primarily a filter feeder (Stephenson, 1935). Casual observations in aquaria in the laboratory tend to confirm these arguments. So it seems most likely that a newly produced polyp is independent of clonemates, and that the environment acts independently upon each member of a clone.

That is not to say, however, that asexual reproduction is not an important adaptive strategy. Williams (1975) has likened ameiotically-produced offspring to multiple copies of the same lottery ticket and argued that under certain conditions sexual reproduction serves to increase the chances that an individual's offspring will contain a winning combination. This can account for the presence of sexual reproduction in an organism with asexual capacities in the face of the fifty per cent genetic cost of sexual reproduction. Balancing this, however, the presence of an active organism, such as a polyp of *Metridium*, implies that a winning combination has been produced (evidenced by the success of the individual) and that the environment occupied is suited at least to the maintenance of the individual. It may, in this case, be advantageous to reproduce multiple copies of the same "ticket," since it has already proved that it is a winner. It is also possible to argue that large clones increase the chances of a genotype making a contribution

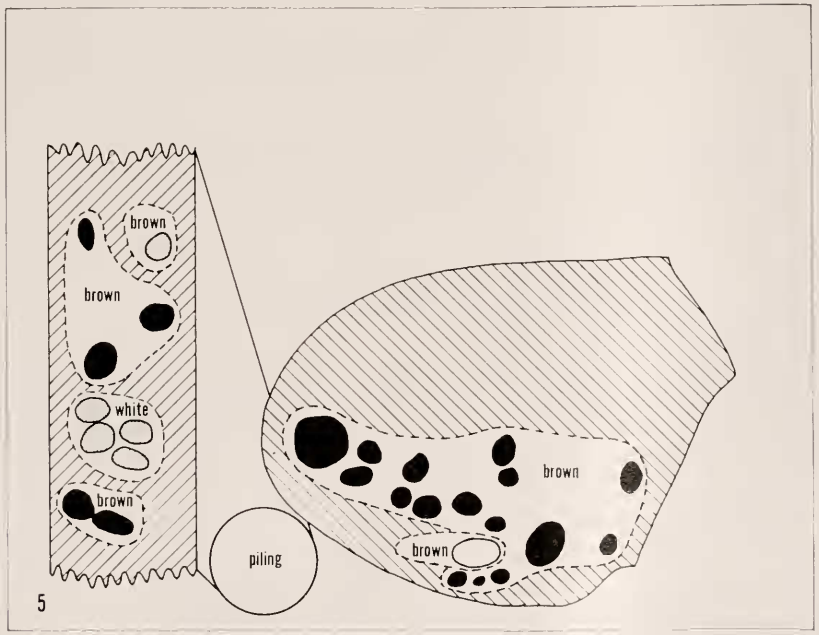


FIGURE 5. Distribution of clones and genotypes at a collecting station in Barnstable Town Boat Harbor. Symbols as in Figures 1–4.

to the next sexual generation. By asexual reproduction it is possible to duplicate the genome without the genetic cost of sexual reproduction.

None of Williams' models of sexual and asexual reproduction seems to fit the *Metridium* case adequately, since they require association between siblings (Williams, 1975, p. 44) to explain the existence of sexual reproduction at all in animals of this type. Such association is extremely unlikely in a planktonically-dispersed organism like *Metridium* (or, indeed, like corals, which, ironically, do not fit his Strawberry-Coral model). Williams' models predict intense selection in organisms of this type, but there is no evidence for this with respect to PHI at least. All gentoypes enjoy some success.

It is tempting to conclude that there is selection for the heterozygotes of PHI at Barnstable. While simple excess of heterozygotes over expectations does not necessarily imply maintenance of the polymorphism by overdominance, there is no significant change in gene frequency during the production of the heterozygote excess (Table I); this would constitute evidence for heterosis as the mechanism maintaining the polymorphism (Lewontin, 1974, pg. 242), assuming that a significant change in gene frequency could be detected with these sample sizes. In fact, the detection of a significant departure from Hardy-Weinberg expectations is a remarkable result, since the departure was detected using a very weak statistical test (see Ward and Sing, 1970 and Lewontin, 1974, for discussion of the lack of power of the χ^2-test to detect departures from Hardy-Weinberg equilibrium with manageable sample sizes). However, as previously stated, the fact that newly established polyps do not deviate from Hardy-Weinberg equilibrium and the polyps that make up the resulting clones do deviate does not necessarily imply that these two groups differ significantly from one another. In fact, as shown above, the genotype distributions of these two groups do not differ significantly from each other. The conclusion of heterozygote superiority must remain speculative.

There is, however, other evidence that PHI genotype may contribute to the success of a polyp at asexual reproduction at Barnstable. There may be a tendency for s/s polyps to produce small clones, since the s/s clones are constituted of one (Figure 5) and three (Figure 2) polyps each. This conclusion would be strengthened by a larger sample size, since only two s/s clones were discovered at Barnstable. Further, the occurrence of the large s/s aggregation at Cape Cod Canal raises the question of whether this result is due to sampling or to locally different selective regimes.

While the lack of mapping and exhaustive collections at Cape Cod Canal and Woods Hole may cloud the issue, there may also be evidence for local differentiation of the three populations. G-test statistics reveal marked heterogeneity of genotype distribution among the three locations, regardless of whether station B-2 from Cape Cod Canal is included in the analysis or not. Unfortunately, without detailed clonal analysis, it is impossible to tell whether the heterogeneity is due to the sampling of several animals from the same clones at a location (meaning that samples at a given location may not be truly independent) or to real differences in gene frequency.

Although this study seems to show tentative evidence that PHI contributes to fitness during asexual reproduction, it is not clear how selection might be operating,

Is the polymorphism maintained by heterosis, or is there a linkage effect that reduces the apparent heterosis to a marker effect for some other locus that is heterotic? Is there some functional disadvantage to the s/s genotype? If so, what balances it? Is balancing selection involved at all? These questions must remain unanswered until data can be obtained on the catalytic properties of the alternative alleles and the heterozygote mixture. Several enzymes show optimum substrate binding only at temperatures experienced by the organism (Somero, 1969; Somero and Hochachka, 1971). It could be that heterozygosity allows optimum binding of substrate over a broader range of temperatures than is possible with only a single enzyme species. Temperature certainly is variable for *Metridium* in the Woods Hole region, annually ranging from 1.8° C to 21.8° C (Sassaman and Mangum, 1970), an important consideration for a sessile animal that cannot escape the variability. But this must remain speculative until data can determine whether it is realistic. Only by accumulating such mechanistic information can the mode of action of selection on this and other enzyme polymorphisms be understood. The investigation of the kinetic properties of PHI variants in *Metridium* will be the subject of continuing investigation in this laboratory.

It is clear, nonetheless, that studies of sessile, asexually reproducing organisms provide powerful tools for the examination of the dynamics of genetic change in natural populations. Not only is it possible to document the distribution of genotypes at some point in the past by inferences about the genotypes of the founders of clones, but it is also possible to measure the success of a given genotype in occupying ecological space by the production of large clones. The resolution possible in this kind of study will be improved with the addition of other polymorphic loci.

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Summary

1. *Metridium senile* was studied for phosphohexose-isomerase variation at three locations on Cape Cod, Massachusetts: Woods Hole, Cape Cod Canal, and Barnstable Town Boat Harbor.

2. All three locations exhibited significant polymorphism for PHI.

3. Mapping of individual polyps was performed at Barnstable to analyze spatial distributions of clones and genotypes.

4. In Barnstable, PHI does not depart significantly from Hardy-Weinberg expectations at the time of establishment of new polyps, and establishment of larvae is spatially random with respect to PHI genotype.

5. Asexual reproduction was used as a measure of the relative success of different PHI genotypes. There are indications that not all genotypes are equally likely to produce large clones.

6. There is significant heterogeneity among the three locations with respect to PHI genotype frequencies, suggesting that there may be geographical differentiation of the populations.

7. Sessile, asexual organisms provide powerful tools for examining the dynamic aspects of genetic structure in natural populations.

LITERATURE CITED

Boyer, J. F., 1974. Clinal and size-dependent variation in the LAP locus in *Mytilus edulis*. *Biol. Bull.*, **147**: 535-549.

Campbell, R. D., 1974. Cnidaria. Pages 133–199 in A. C. Giese and J. S. Pearse, Eds., *Reproduction of marine invertebrates, Volume I.* Academic Press, New York.

Costello, D. P., M. E. Davidson, A. Eggers, M. H. Fox, and C. Henley, 1957. *Methods for obtaining and handling marine eggs and embryos.* Marine Biological Laboratory, Woods Hole, 247 pp.

Dobzhansky, T., 1970. *Genetics of the evolutionary process.* Columbia University Press, New York, 505 pp.

Francis, L., 1973a. Clone specific segregation in the sea anemone *Anthopleura elegantissima*. *Biol. Bull.*, **144**: 64–72.

Francis, L., 1973b. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. *Biol. Bull.*, **144**: 73–92.

Fox, D. L., and C. F. A. Pantin, 1941. The colours of the plumose anemone, *Metridium senile* (L.). *Phil. Trans. Roy. Soc. London Ser. B*, **230**: 415–450.

Fox, D. L., G. F. Crozier, and V. E. Smith, 1967. Carotenoid fractionation in the plumose anemone *Metridium*. *Comp. Biochem. Physiol.*, **22**: 177–188.

Gemmill, J. F., 1920. The development of the sea-anemones *Metridium dianthus* (Ellis) and *Adamsia palliata* (Bohad). *Phil. Trans. Roy. Soc. London Ser. B*, **209**: 351–375.

Hahn, C. W., 1905. Dimorphism and regeneration in *Metridium*. *J. Exp. Zool.*, **2**: 225–235.

Koehn, R. K., and J. B. Mitton, 1972. Population genetics of marine pelecypods. I. Ecological heterogeneity and evolutionary strategy at an enzyme locus. *Amer. Natur.*, **106**: 47–56.

Koehn, R. K., and F. J. Turano, and J. B. Mitton, 1973. Population genetics of marine pelecypods. II. Genetic differences in microhabitats of *Modiolus demissus*. *Evolution*, **27**: 100–105.

Lewontin, R. C., 1974. *The genetic basis of evolutionary change.* Columbia University Press, New York, 346 pp.

Sassaman, C., and C. P. Mangum, 1970. Patterns of temperature adaptation in North American Atlantic coastal actinians. *Mar. Biol.*, **7**: 123–130.

Selander, R. K., W. G. Hunt, and S. Y. Yang, 1969. Protein polymorphism and genic heterozygosity in two European subspecies of the house mouse. *Evolution*, **23**: 379–390.

Sokal, R. R., and F. J. Rohlf, 1969. *Biometry.* W. H. Freeman and Co., San Francisco, 776 pp.

Somero, G. N., 1969. Enzymic mechanisms of temperature compensation: immediate and evolutionary effects of temperature on enzymes of aquatic poikilotherms. *Amer. Natur.*, **103**: 517–530.

Somero, G. N., and P. W. Hochachka, 1971. Biochemical adaptation to the environment. *Amer. Zool.*, **11**: 150–167.

Stephenson, T. A., 1935. *The British sea anemones, Vol. II.* The Ray Society, London, 426 pp.

Torrey, H. B., 1902. Papers from the Harriman Alaska expedition. XXX. Anemones, with a discussion of variation in *Metridium*. *Proc. Wash. Acad. Sci.*, **4**: 373–410.

Tracey, M. L., N. F. Bellet, and C. D. Gravem, 1975. Excess allozyme homozygosity and breeding structure in the mussel *Mytilus californianus*. *Mar. Biol.*, **32**: 303–311.

Ward, R. H., and C. F. Sing, 1970. A consideration of the power of the χ^2 test to detect imbreeding effects in natural populations. *Amer. Natur.* **104**: 355–365.

Wilkins, N. P., and N. F. Mathers, 1974. Phenotypes of phosphoglucose isomerase in some bivalve molluscs. *Comp. Biochem. Physiol.*, **48B**: 599–611.

Williams, G. C., 1975. *Sex and evolution.* Princeton University Press, Princeton, 200 pp.